Supporting Information

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**SI Materials and Methods**

**Microscopy.** Monodansylcadaverine (MDC)-stained roots were visualized in vivo using a Zeiss Axioplan II compound microscope (Carl Zeiss). MDC-stained suspension cells, protoplasts, and tissues were visualized using a DAPI-specific filter. The SYTO RNASelect work and some CFP/YFP work were conducted using a Leica SP5× confocal microscope (Leica Microsystems). The SYTO RNASelect green stain was excited using a 490-nm laser and observed using an emission bandwidth of 515–540 nm. Chlorophyll fluorescence was excited using a 490-nm laser and observed using an emission bandwidth of 651–704 nm. CFP fluorescence was excited using a 405-nm UV Laser and observed using an emission bandwidth of 450–505 nm. YFP fluorescence was excited using the 514-nm laser line of the Argon laser and observed using an emission bandwidth of 525–600 nm. The majority of the CFP/YFP work was conducted using a Nikon C1si confocal microscope (Nikon Instruments) with similar settings. Metamorph Line Scan Analysis was performed on confocal fluorescence images to measure the average fluorescent signal. A total of 30 roots were measured for the 7-d-old WT and rns2-2 seedlings.

**Protoplast Preparation.** Mesophyll protoplasts were prepared from leaves using a modified version of the method described by Yoo et al. (1). The concentration of cellulase (Yakult Pharmaceuticals) was 0.1%, and the concentration of macerozyme (Yakult Pharmaceuticals) was 0.1%. Vacuum infiltration was not used, and digestion took place for 4 h at room temperature in the dark. Cells were maintained in W5 buffer (154 mM NaCl, 125 mM CaCl2, 5 mM KCl, 2 mM MES [2-(N-morpholino)ethanesulfonic acid] buffer, pH 5.7) in darkness at room temperature with 40 rpm orbital rotation.


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**Fig. S1.** Preparation of an active CFP-RNS2 fusion. (A) Schematic of the construct used to obtain the CFP-RNS2 fusion protein used in localization studies. 35Sp, CaMV 35S promoter; SP, signal peptide; ER, putative endoplasmic reticulum retention signal. (B) Upper) RNase activity in gel assay of protein extracts obtained from independent plant lines transformed with the CFP-RNS2 construct (#1–#5), plants transformed with CFP alone (CFP), or untransformed wild-type (WT) plants. The position of RNS2 was determined by comparing WT plants and plants lacking RNS2 activity (see Fig. 4). The predicted position of the CFP-RNS2 protein is also indicated. (B Lower) Protein extracts were analyzed by SDS/PAGE and stained with Coomassie Blue R-250 to control for equal loading and protein integrity. Each lane in both gels contains 40 μg of protein. Results are representative of at least five independent experiments.
Fig. S2. RNS2 is a glycosylated protein. Arabidopsis leaf extracts were incubated at 37 °C for 1 or 4 h with or without the addition of N-glycosydase F. After the incubation, the extracts were analyzed in an RNase activity in gel assay. The position of RNS2 is indicated (RNS2-glyc., glycosylated form of RNS2; RNS2, de-glycosylated form). The white box shows the position of the fusion protein CFP-RNS2. (Inset) The image was color-inverted and adjusted for contrast to enhance the visibility of the bands.

Fig. S3. Subcellular localization of CFP-RNS2. Protoplasts of plants expressing CFP-RNS2 (A and B) or WT (C) were imaged using confocal microscopy. (Left panels) Images obtained at the CFP emission wavelength. Center panels correspond to YFP (no signal because these plants do not express the YFP-HDEL ER marker). (Right panels) Bright-field images. These images serve as controls for the images shown in Fig. 2 of the main text.
Fig. S4. Subcellular localization of CFP-RNS2. Roots of plants expressing CFP-RNS2 (A and B) or WT (C) were imaged using confocal microscopy. (A and B) Localization of CFP signal in vacuoles and ER bodies, respectively. Image of WT plant (C) was obtained with the same settings used for B. (Scale bar, 40 μm.)
Fig. S5. RNS2 Genomic DNA, coding region and encoded protein, and position of the T-DNA insertion in the rns2-2 mutant.

Fig. S6. Decay of rRNA in wild-type plants and in lines with altered levels of RNS2 expression. One-week-old seedlings grown in liquid medium were incubated with $^{3}$H-uridine for 30 min and then transferred to cold medium. At the indicated time points, samples were extracted and total RNA was isolated. To determine the fraction of $^{3}$H-RNA versus total RNA for the 18S rRNA, the samples were analyzed by agarose gel electrophoresis and transferred to nitrocellulose. Total 18S rRNA was determined by ethidium-bromide staining, then bands were excised, and the tritium signal was quantified with a scintillation counter. (A) Comparison of the decay of 18S rRNA in WT and the rns2-2 mutant. (B) 18S rRNA decay in WT and in a RNS2 antisense line (AS-RNS2). Asterisks indicate statistically significant differences with respect to WT ($P < 0.01$). Although the difference between WT and AS-RNS2 is not significant due to large variability of the AS-RNS2 sample, the observed trend is similar to that of rns2-2. Asterisk indicates statistically significant differences ($P < 0.05$) with respect to WT.
Fig. S7. Loss of RNS2 activity leads to constitutive autophagy. Autophagosomes were detected by MDC staining of root tips. One-week-old wild-type (WT, Left panels), rns2-2 (Center panels), and AS-RNS2 (Right panels) seedlings were transferred to fresh solid MS medium with or without sucrose in the dark for 4 d, followed by staining with MDC. Bright-field and MDC fluorescence are shown. (Scale bar, 50 μm.)